

Mitochondrial DNA Variation of Modern Tuscans Supports the Near Eastern Origin of Etruscans

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The origin of the Etruscan people has been a source of major controversy for the past 2,500 years, and several hypotheses have been proposed to explain their language and sophisticated culture, including an Aegean/Anatolian origin. To address this issue, we analyzed the mitochondrial DNA (mtDNA) of 322 subjects from three well-defined areas of Tuscany and compared their sequence variation with that of 55 western Eurasian populations. Interpopulation comparisons reveal that the modern population of Murlo, a small town of Etruscan origin, is characterized by an unusually high frequency (17.5%) of Near Eastern mtDNA haplogroups. Each of these haplogroups is represented by different haplotypes, thus dismissing the possibility that the genetic allocation of the Murlo people is due to drift. Other Tuscan populations do not show the same striking feature; however, overall, ~5% of mtDNA haplotypes in Tuscany are shared exclusively between Tuscans and Near Easterners and occupy terminal positions in the phylogeny. These findings support a direct and rather recent genetic input from the Near East—a scenario in agreement with the Lydian origin of Etruscans. Such a genetic contribution has been extensively diluted by admixture, but it appears that there are still locations in Tuscany, such as Murlo, where traces of its arrival are easily detectable.

The Etruscan culture developed and prospered in the 9th century B.C. over a region in central Italy named “Etruria”—usually referred to in Greek and Latin sources as “Tyrrhenia.” Etruria encompassed what is now known as “Tuscany,” “northern Latium,” and “western Umbria.” The culture of the Etruscan people was not only extremely advanced but also distinctively different from the cultures of the surrounding populations of Italy. Because of this, the origin of the Etruscan people has been a source of controversy for the past 2,500 years, with two major scenarios being disputed. The first is that the Etruscans were an autochthonous population of Italy; the alternative hypothesis is that they were direct descendants of an immigrant group of Aegean/Anatolian ancestry.¹

Etruria was dominant in the Italian peninsula after 650 B.C., when Etruscans began to expand toward both the north (the Po River Valley) and the south. Etruscan kings conquered and ruled Rome for 100 years, until 509 B.C., when the last Etruscan king, Lucius Tarquinius Superbus, was removed from power and the Roman Republic was established. From that moment, the expansion period of the Etruscans ended and was followed by a slow process of conquest and assimilation by the Romans, which cul-

minated at the time of the “Social War” (90–88 B.C.), with the attribution of Roman citizenship to all Etruscans. Rapidly, the Etruscan culture and language disappeared,^{2,3} possibly also because, in the following decades, Etrurian lands were often distributed to Roman veterans and were partially repopulated by poor Roman citizens. However, despite a potentially extensive dilution of the ancestral Etruscan gene pool with that from surrounding Italic populations, there is no evidence that such a gene pool had been completely replaced.⁴ In addition, part of the process of initial assimilation might have been mainly male mediated, since incoming Roman veterans often married women from local communities. Thus, some populations of modern Tuscany should have retained at least a fraction of such an ancestral gene pool—particularly the exclusively maternally inherited mtDNA pool—possibly at a variable extent, given the differential degree of geographical and genetic isolation of the different Tuscan communities during the past 25 centuries.

To evaluate the nature and extent of mtDNA variation in modern Tuscans, the mtDNA from 322 subjects from three areas of Tuscany was analyzed: 86 from Murlo, a rather isolated town of Etruscan origin in the Siena prov-

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Table 1. Frequencies of mtDNA Haplogroups in the Three Tuscan Populations

Haplogroup ^a	No. (%) of Subjects by Population			
	Murlo (<i>N</i> = 86)	Volterra (<i>N</i> = 114)	Casentino Valley (<i>N</i> = 122)	Total (<i>N</i> = 322)
H:	27 (31.4)	53 (46.5)	46 (37.7)	126 (39.1)
H*	14 (16.3)	31 (27.2)	15 (12.3)	60 (18.6)
H1	4 (4.7)	13 (11.4)	19 (15.6)	36 (11.2)
H3	5 (5.8)	5 (1.6)
H5	3 (3.5)	3 (2.6)	8 (6.6)	14 (4.3)
H6	1 (1.2)	1 (.9)	...	2 (.6)
H11	...	2 (1.8)	1 (.8)	3 (.9)
H14	...	1 (.9)	...	1 (.3)
H15	2 (1.6)	2 (.6)
H19	...	2 (1.8)	1 (.8)	3 (.9)
HV0:	5 (5.8)	7 (6.1)	5 (4.1)	17 (5.3)
HV0*	1 (1.2)	3 (2.6)	1 (.8)	5 (1.6)
HV0a	1 (1.2)	1 (.9)	...	2 (.6)
V	3 (3.5)	3 (2.6)	4 (3.3)	10 (3.1)
HV (without H/HV0):	6 (7.0)	3 (2.6)	7 (5.7)	16 (5.0)
HV*	5 (5.8)	3 (2.6)	6 (4.9)	14 (4.3)
HV1	1 (1.2)	...	1 (.8)	2 (.6)
R0:	1 (1.2)	5 (4.4)	2 (1.6)	8 (2.5)
R0a	1 (1.2)	5 (4.4)	2 (1.6)	8 (2.5)
R2:	...	1 (.9)	...	1 (.3)
R2*	...	1 (.9)	...	1 (.3)
J:	7 (8.1)	7 (6.1)	4 (3.3)	18 (5.6)
J1	6 (7.0)	5 (4.4)	2 (1.6)	13 (4.0)
J2	1 (1.2)	2 (1.8)	2 (1.6)	5 (1.6)
T:	8 (9.3)	15 (13.2)	13 (10.7)	36 (11.2)
T1	1 (1.2)	3 (2.6)	4 (3.3)	8 (2.5)
T2	7 (8.1)	12 (10.5)	9 (7.4)	28 (8.7)
U:	26 (30.2)	14 (12.3)	29 (23.8)	69 (21.4)
U1	1 (1.2)	1 (.9)	...	2 (.6)
U2e	5 (5.8)	1 (.9)	1 (.8)	7 (2.2)
U3	5 (5.8)	1 (.9)	3 (2.5)	9 (2.8)
U4	1 (1.2)	4 (3.5)	1 (.8)	6 (1.9)
U5a	3 (3.5)	2 (1.8)	2 (1.6)	7 (2.2)
U5b	6 (4.9)	6 (1.9)
U6a	1 (1.2)	...	1 (.8)	2 (.6)
U7	4 (4.7)	1 (.9)	3 (2.5)	8 (2.5)
K1	6 (7.0)	4 (3.5)	12 (9.8)	22 (6.8)
N1:	...	4 (3.5)	2 (1.6)	6 (1.9)
N1b1	1 (.8)	1 (.3)
N1c	...	1 (.9)	1 (.8)	2 (.6)
I	...	3 (2.6)	...	3 (.9)
N2:	1 (1.2)	...	7 (5.7)	8 (2.5)
N2a	1 (.8)	1 (.3)
W	1 (1.2)	...	6 (4.9)	7 (2.2)
X:	3 (3.5)	2 (1.8)	3 (2.5)	8 (2.5)
X2	3 (3.5)	2 (1.8)	3 (2.5)	8 (2.5)
M:	1 (1.2)	...	2 (1.6)	3 (.9)
M1	2 (1.6)	2 (.6)
D4g1	1 (1.2)	1 (.3)
Sub-Saharan L:	1 (1.2)	3 (2.6)	2 (1.6)	6 (1.9)
L3d	1 (1.2)	3 (2.6)	1 (.8)	5 (1.6)
L1b	1 (.8)	1 (.3)

^a HV0, HV0*, HV0a, R0, and R0a replace pre-V, pre*V1, pre*V2, pre-HV, and (pre-HV)1, respectively.

Table 2. Absolute Frequencies of mtDNA Haplogroups and Subhaplogroups in the 58 Populations Included in the PCA

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

ince; 114 from Volterra, a former major Etruscan city in the province of Pisa; and 122 from the Casentino Valley in the province of Arezzo, which was part of historical Etruria.

mtDNA profiles in the three populations were determined by sequencing ~750 bp from the control region for each subject (from nucleotide position [np] 16024 to np 210, thus including the entire hypervariable segment [HVS]-I [nps 16024–16383] and part of the HVS-II [nps 57–372]). This was followed by a hierarchical survey of haplogroup and subhaplogroup diagnostic markers in the coding region,⁵ which allowed the classification of mtDNAs into 39 haplogroups (table 1). Most haplogroups that are typical of modern European populations are present,^{6–8}

and a few East Asian (D4g1)⁹ and sub-Saharan African (L3d and L1b)¹⁰ mtDNAs were also detected. This latter finding is not unexpected, since low frequencies of African and even East Asian mtDNAs are not uncommon in populations of southern Europe. However, what was not anticipated was the relatively high frequency, especially in the Murlo sample, of haplogroup HV lineages that are non-H and non-HV0, as well as haplogroups R0a (formerly known as “(pre-HV)1”)¹¹ and U7 and U3, which are typical of Near Eastern populations.

To determine whether the overall haplogroup frequencies in the Tuscan population samples were indeed different from those of other Italian or European populations, we compared haplogroup distributions in the three Tuscan populations (table 1) with those of 55 western Eurasian populations (including 7 from other Italian regions) (table 2), by using principal-component analysis (PCA)—a dimension-reduction method that seeks to explain the variance of multivariate data by a smaller number of variables (the principal components [PCs]), which are linear func-

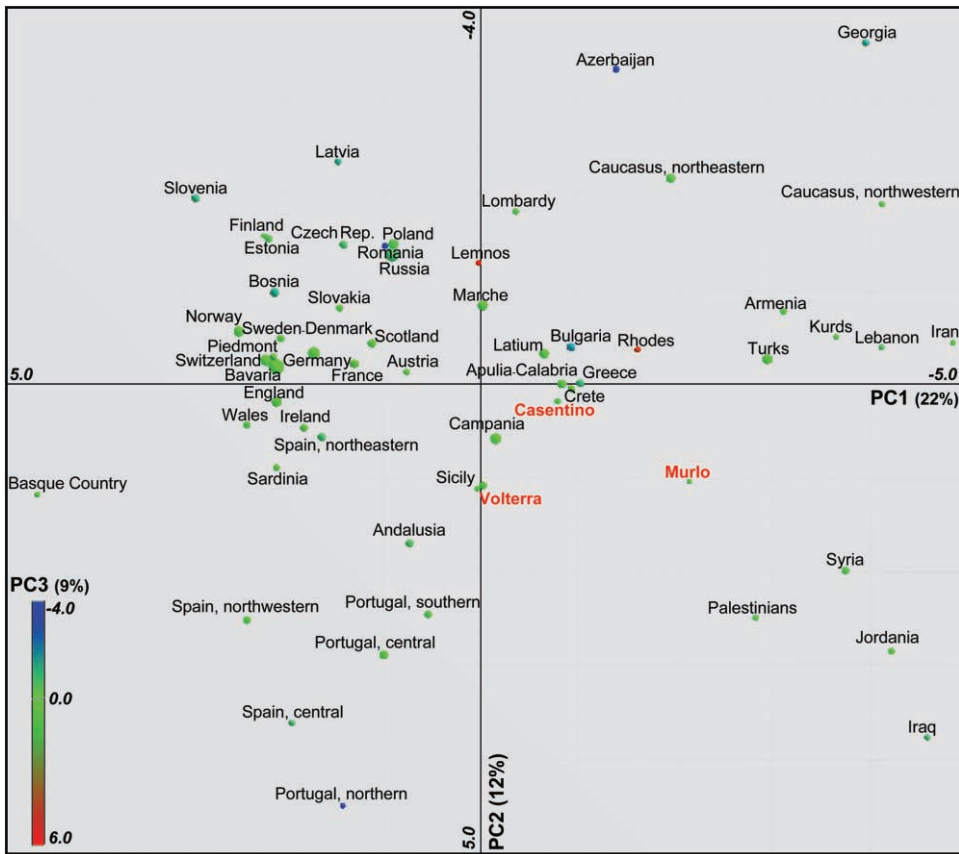


Figure 1. Four-dimensional region-based PCA of mtDNA haplogroup profiles in Europe and the Near East. Haplogroups and subhaplogroups included in the PCA are as follows: H (excluding H5), H5, HV0 (including HV0*, HV0a, and V), HV (including HV1 but excluding H and HV0), R0a, U1, U2, U3, U4, U5a, U5b, U6, U7, U8, K, U* (represented mainly by U9), J, T1, T2, X, N1 (represented mainly by I), N2 (represented mainly by W), M, and sub-Saharan L. The size of the circles represents the remaining axes (4–24) and is inversely proportional to the error associated with the projection of each population on the first three PCs.¹³ The numbers of subjects and haplogroup frequencies for each population are provided in table 2.

tions of the original variables—in this case, the haplogroup frequencies.¹² After reducing the variables (haplogroups) to PCs, we reported the coordinates of the observations for the 58 populations in a four-dimensional graphic representing the genetic landscape of western Eurasia (fig. 1). We observed that the first PC is primarily east-west, separating Europeans from Near Easterners, and it accounts for 22% of the variation. The Basque country falls at one pole, Iran at the other. Such a strong geographical pattern, with the Near Easterners forming a well-defined group and the eastern and central Mediterranean populations of Europe as their most closely related neighbors, is in perfect agreement with the patterns reported elsewhere for both classical¹² and mtDNA and Y-chromosome markers.¹⁴ The second PC accounts for 12% of the variation and appears to be determined mainly by the variation within the Near East and the Caucasus on one side, whereas, on the European side, it reveals a southwest-northeast pattern, with Iberian populations at one end and northeastern Europe at the other.

As for the population samples from Tuscany, both the first and second PC place Volterra and the Casentino Valley in the central Mediterranean area, together with most of the other Italian samples. In contrast, Murlo is placed relatively close to the Near Eastern populations. This peculiar location of Murlo is also maintained overall by including additional PCs (fig. 1). Interestingly, the third PC indicates that the modern population of Lemnos is an outlier in the genetic landscape, with particular features distinguish-

ing it from both modern European and Near Eastern populations. It is worth mentioning (1) that Lemnos is the island in the northern Aegean Sea that was indicated by Hellanicus of Lesbos (5th century B.C.) as a possible homeland of a group of Pelasgians who arrived in Italy and gave rise to the Tyrrhenians and (2) that a stele—dated ~600 B.C.—found on Lemnos contains inscriptions in a language similar to Etruscan.¹⁵ However, like many small islands, Lemnos must have undergone much more drift than did villages like Murlo that are located in a densely populated region, as well as many more warring events.

The contribution of each haplogroup to the first and second PC, in the analysis shown in figure 1, is illustrated in figure 2. Haplogroups H (in western Europe, comprising mainly H1 and H3), HV0 (formerly known as “pre-V”), and U5b are concentrated at the European pole, which confirms the major role played by the Franco-Cantabrian refuge area in the recolonization of Europe after the Last Glacial Maximum.¹⁶ At the other pole—the one where Murlo is located, with the populations from the Near East—the remainder (HV* plus HV1) of haplogroup HV and haplogroups R0a, U7, and U3 are the major contributors, and the absolute frequencies of three of these (HV without H and HV0, R0a, and U7) differ significantly ($P < .01$) when Tuscans, Europeans (not including Tuscans), and non-Europeans are compared (table 3). Moreover, in contrast to in Europeans (not including Tuscans), the frequencies of these three haplogroups in Tuscans and non-

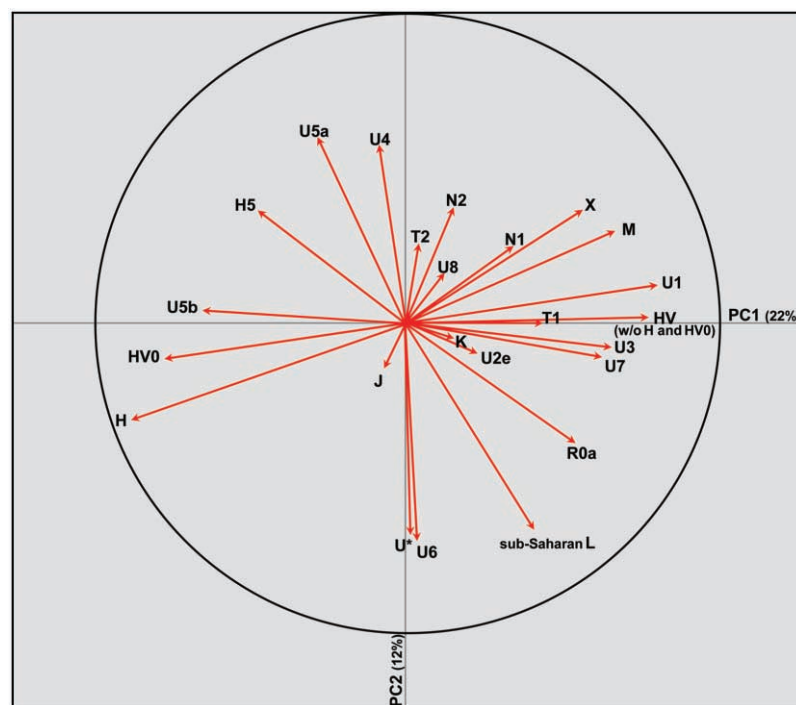


Figure 2. Plot of the contribution of each haplogroup to the first and second PC (projections of the axes of the original variables) in the analysis of figure 1.

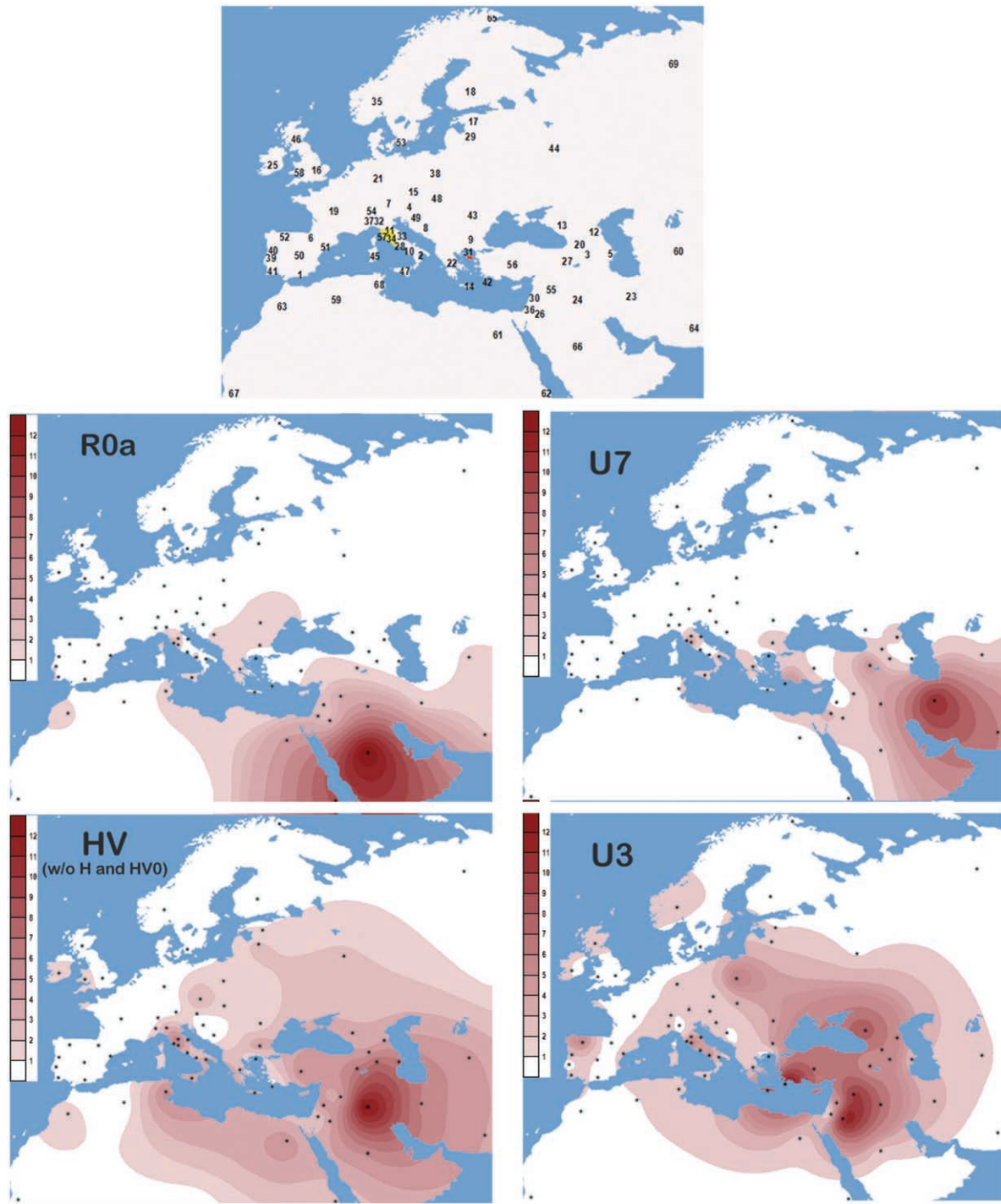


Figure 3. Geographical locations of populations surveyed for R0a, HV (without H and HV0), U7, and U3 mtDNAs and their spatial frequency distributions. Frequency maps of haplogroups were obtained using Surfer version 6.04 (Golden Software), with the Kriging procedure, and estimates at each grid node were inferred by considering the entire data set. Etruria and the island of Lemnos (*top*) are indicated in yellow and red, respectively. Frequency values for populations 1–58 are from table 2, whereas those for populations 59–69 are as follows: 59 from the present study and the work of Plaza et al.¹⁷; 60 and 64 from the work of Quintana-Murci et al.¹⁸; 61 from the present study and the work of Richards et al.⁷; 62, 65–67, and 69 from the present study; 63 from the present study and the work of Plaza et al.¹⁷; and 68 from the work of Fadhlouli-Zid et al.¹⁹

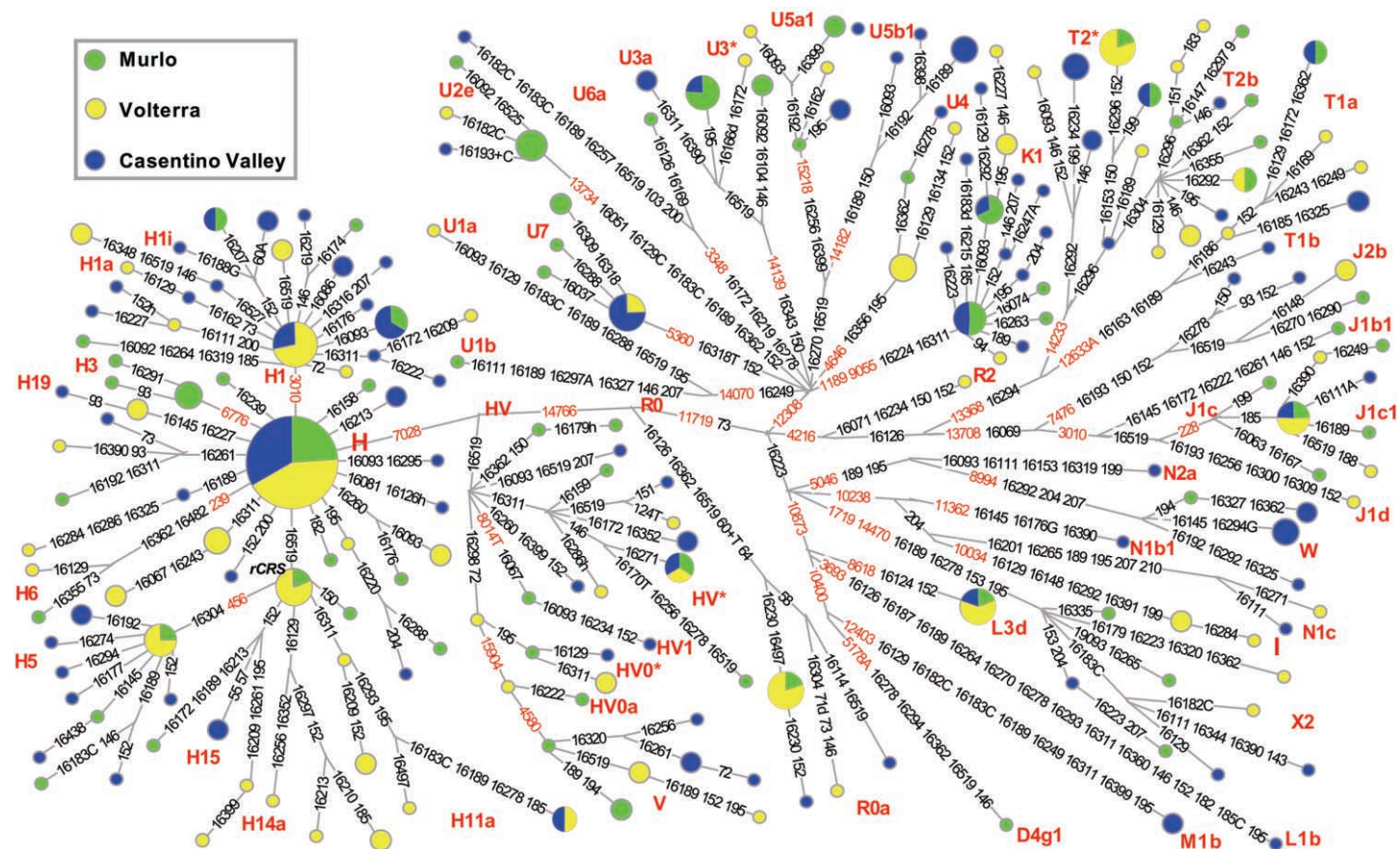


Figure 4. Tree of the 209 mtDNA haplotypes observed among the 322 Tuscans (GenBank accession numbers EF026248–EF026569), subdivided according to their origin. This tree was constructed manually by comparison with the available mtDNA data sets and the basal Eurasian mtDNA classification trees.^{8–11,16} Haplotypes (from np 16024 to np 210) are 53 from Murlo, 59 from Volterra, 78 from the Casentino Valley, and 19 that are shared by at least two of the Tuscan groups. Haplogroups and subhaplogroups are indicated in red. The numbers on the connecting branches refer to the revised reference sequence (rCRS)²⁰ and indicate either mutations from the sequence range or haplogroup diagnostic sites (red) outside that range. Mutations are transitions unless the base change is explicitly indicated. Insertions are suffixed with a plus sign (+) and the inserted nucleotide, and deletions have a “d” suffix. Heteroplasmic positions are indicated by an “h” after the nucleotide position.

Figure 5. Haplotypes found in Tuscany and shared exclusively with Near Eastern populations. Shared haplotypes are shown in different colors. Near East areas where the matching haplotypes were detected are illustrated on the map (*inset*) by colored circles. A group of six related R0a mtDNAs with the basic motif 16126-16230-16362, as well as two HV mtDNAs with the motif 16172-16311-16352, were found only in Tuscans (*black*). For additional information, see the legend of figure 4.

Table 3. χ^2 Analysis of Absolute Frequencies in Tuscans, Europeans (without Tuscans), and Non-Europeans

Comparison	χ^2 Value (df)	<i>P</i> ^a
Tuscans and Europeans	144 (24)	<.01
Europeans and non-Europeans	2,070 (24)	<<.01
All three groups	2,144 (48)	<<.01

^a Statistical significance was established at $\alpha \leq .05$; the *P* values were adjusted for multiple tests by use of the (conservative) Bonferroni correction.

Europeans are both higher than expected (table 4). The spatial distribution of haplogroups HV without H and HV0, R0a, U7, and U3 in an overall frequency map that encompasses western Eurasia and North Africa is illustrated in figure 3. This figure reveals that, for all of these haplogroups, there is a frequency peak in the Near East, thus explaining the major contributing role of these four haplogroups in the PCA. However, in all cases, Tuscany was found to harbor a secondary frequency peak, which often neatly distinguishes this Italian region not only from the rest of Europe but also from the surrounding regions of the Italian peninsula. Haplotype diversity (fig. 4) indicates that these peaks—in contrast to those observed, for instance, in northeastern Spain for U3 or in Ireland and Wales for HV (fig. 3)—cannot be attributed to genetic drift and/or founder events.

After evaluating interpopulation relationships at the haplogroup level, we moved to a different level of resolution—that of haplotypes. We compared the 209 control-region haplotypes found in the three Tuscan groups, whose phylogenetic relationships are illustrated in the trees of figures 4 and 5, with a data set of 15,328 control-region sequences that are representative of a wide range of western Eurasian populations (table 2). This survey revealed 11 haplotypes/motifs (~5%) (in color in the tree of fig. 5) that belong to different haplogroups (H, R0a, U1b, U7, J1d, and T1a) that are shared between Tuscans and Near Eastern populations but are absent in a total of 10,589 mtDNAs from Europe, including 2,311 mtDNAs from Italy. All others, except two related R0a haplotypes and one HV haplotype that were seen only in Tuscans (fig. 5), were shared either with at least one European population or with both European and Near Eastern populations. One of the H motifs (16145–16227), shared exclusively between Tuscans (two from Volterra and one from the Casentino Valley) and Near Easterners (one Turk, one Jordanian, and one Syrian), appears to define a novel subhaplogroup, named “H19.” The corresponding six HVS-I sequences sharing this motif are identical. This suggests that, if the motif 16145–16227 arose in the Near East, its arrival in Tuscany is recent. Similarly, ages of the other 10 shared haplotypes cannot be reasonably estimated. However, all occupy terminal positions in the tree of figure 5, indicating a rather recent occurrence among Tuscans.

It is worth noting that such an extent of exclusive haplotype sharing between Tuscans and Near Eastern popula-

tions is more than threefold higher than that observed in a total of 314 HVS-I haplotypes from the Marche region—a neighboring region of central Italy located just across the Apennines—which, however, was never inhabited or conquered by the Etruscans.

The extensive haplotype sharing between Tuscans and other western Eurasian populations—including a detectable amount with only Levantine populations—strongly contrasts with the results obtained by comparing the haplotypes found in Tuscans with the 23 haplotypes detected in the skeletal remains of 28 Etruscans.²¹ The latter comparison reveals only three matches (CRS, 16261, and 16311). When the Etruscan haplotypes are instead compared with 2,311 Italian mtDNAs, seven matches are observed (CRS, 16126, 16129, 16223, 16261, 16311, and 16189–16356), apparently reaching a plateau, since they remain the same when the counterpart is represented by 15,328 western Eurasian mtDNAs. Thus, ~70% of the recorded Etruscan haplotypes do not fit anywhere in the mtDNA landscape of western Eurasia—a result that is difficult to explain. Given the fact that the matched haplotypes are the simplest in terms of number of mutations (generally one nucleotide difference from the reference sequence), a likely explanation is that postmortem DNA modifications and/or technical problems^{22,23} affected the Etruscan mtDNA sequences.^{24,25} Such a scenario not only makes it unlikely that models and simulations based on such data²⁶ will provide new clues on the origin of Etruscans, but it also raises the possibility that ancient mtDNA data, unless associated with clear-cut phylogeographic signals,²⁷ may be more misleading than clarifying in studies concerning the prehistory of regional populations.

Analysis of the maternally inherited mtDNA, which, in the case of Etruria, is probably the most appropriate tool for evaluation of genetic continuity between Etruscans and modern Tuscans, places Murlo close to Near Eastern populations because of an unusually high frequency (17.5%) of Near Eastern haplogroups (HV, R0a, U7, and

Table 4. Results of “ χ^2 by Cell” Test Comparing All Three Groups

Population	Haplogroup		
	HV	R0a	U7
Tuscans	+	+	+
Europeans (without Tuscans)	–	–	–
Non-Europeans	+	+	+

NOTE.—The “ χ^2 by Cell” test was performed using Xlstat software. It is a χ^2 test calculated on a table with four cells: one cell represents cell [i,j] in the original contingency table, the other cells represent frequencies for row i minus cell [i,j], for column j minus cell [i,j], and for the rest of the table. Significance was established at $\alpha \leq .05$, with use of the Bonferroni correction for multiple testing. A plus sign (+) indicates that the observed frequency was higher than the expected frequency; a minus sign (–) indicates that the observed frequency was less than the expected frequency.

U3). Moreover, this allocation cannot be explained by genetic drift, since each of these haplogroups is represented by several different haplotypes. Finally, 5.3% of the haplotypes observed in Tuscany—all occupying terminal positions in the phylogeny—are found elsewhere only in Levantine populations. This distribution suggests a recent and direct link between Tuscany and the Near East—a link not mediated by either geographically intermediate European populations or surrounding Italian populations.

Overall, these mtDNA data and others from different organisms²⁸ support the scenario of a post-Neolithic genetic input from the Near East to the present-day population of Tuscany, a scenario that is in agreement with an Anatolian origin of Etruscans.² Traces of this relatively recent arrival from the Near East are still detectable in Tuscany, despite extensive dilution by admixture with both native and surrounding Italic populations and later immigration.

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Web Resource

Accession numbers and the URL for data presented herein are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for the Tuscan mtDNA control-region sequences [accession numbers EF026248–EF026569])

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